Applicant : Peter Masci Serial No. : 10/667,295

Filed: September 17, 2003

Page : 2 of 18

Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

**Listing of Claims**:

1. (Currently amended) A method for making infertile seed, said method

comprising:

a) permitting seed development to occur on a plurality of first plants that have

been pollinated by a plurality of second plants, wherein said first plants are male-sterile due to

cytoplasmic male sterility, nuclear male sterility or genetic male sterility and comprise first and

second nucleic acids, said first nucleic acid comprising a first transcription activator recognition

site and a first promoter, said first recognition site and said first promoter operably linked to a

sequence to be transcribed, said second nucleic acid comprising a second transcription activator

recognition site and a second promoter, said second recognition site and said second promoter

operably linked to a coding sequence that results in seed infertility,

wherein said second plants are male-fertile and comprise at least one activator nucleic

acid comprising at least one coding sequence for a transcription activator that binds to at least

one of said recognition sites, each said at least one transcription activator coding sequence

having a promoter operably linked thereto, and wherein said seeds are infertile.

2. (Original) The method of claim 1, wherein said at least one activator nucleic

acid is a single nucleic acid encoding a single transcription activator that binds said first and said

second recognition sites.

3. (Original) The method of claim 2, wherein said promoter for said

transcription activator is seed-specific.

Serial No.: 10/667,295

Filed: September 17, 2003

Page : 3 of 18

4. (Original) The method of claim 3, wherein said promoter for said transcription activator is an Arabidopsis LEC1 promoter.

- 5. (Original) The method of claim 2, wherein said promoter for said transcription activator is chemically inducible.
- 6. (Original) The method of claim 1, wherein said at least one activator nucleic acid is a single nucleic acid encoding a first transcription activator that binds said first recognition site and encoding a second transcription activator that binds said second recognition site.
- 7. (Original) The method of claim 6, wherein said promoter for said first transcription activator is a constitutive promoter and said promoter for said second transcription activator is a seed-specific promoter.
- 8. (Original) The method of claim 7, wherein said promoter for said first transcription activator is a maize ubiquitin promoter.
- 9. (Original) The method of claim 1, wherein said plants are dicotyledonous plants.
- 10. (Original) The method of claim 1, wherein said plants are monocotyledonous plants.
- 11. (Original) The method of claim 1, further comprising the step of harvesting said seeds.
- 12. (Original) The method of claim 1, wherein said plurality of first plants is cytoplasmically male-sterile.

Serial No.: 10/667,295

Filed: September 17, 2003

Page : 4 of 18

13. (Currently amended) The method of claim 1, wherein said plurality of first plants is male-sterile due to nucleargenetic male sterility.

- 14. (Original) The method of claim 1, wherein said sequence to be transcribed encodes a preselected polypeptide.
- 15. (Original) The method of claim 14, wherein said seeds have a statistically significant increase in the amount of said preselected polypeptide relative to seeds that do not contain or express said first nucleic acid.
- 16. (Original) The method of claim 15, wherein said preselected polypeptide is an antibody.
- 17. (Original) The method of claim 15, wherein said preselected polypeptide is an enzyme.
- 18. (Original) The method of claim 1, wherein said sequence causing seed infertility encodes a seed infertility polypeptide.
- 19. (Original) The method of claim 18, wherein said seed infertility polypeptide is a loss-of-function mutant FIE polypeptide.
- 20. (Original) The method of claim 18, wherein said seed infertility polypeptide is an ANT polypeptide.
- 21. (Original) The method of claim 18, wherein said seed infertility polypeptide is a LEC1 polypeptide.
- 22. (Currently amended) A method for making a polypeptide, said method comprising:

Serial No.: 10/667,295

Filed: September 17, 2003

Page : 5 of 18

a) obtaining seed produced by pollination of a plant that is male-sterile due to cytoplasmic male sterility, nuclear male sterility or genetic male sterility, said seed comprising:
i) a first nucleic acid comprising a first recognition site for a transcription activator and a first promoter, said first recognition site and said first promoter operably linked to a sequence to be transcribed; ii) a second nucleic acid comprising a second recognition site for a transcription activator and a second promoter, said second recognition site and said second promoter operably linked to a sequence causing seed infertility; and iii) at least one activator nucleic acid comprising at least one coding sequence for a transcription activator that binds to at least one of said recognition sites, each said at least one transcription activator having a promoter operably linked thereto, wherein said seeds are infertile and have a statistically significant increase in the amount of an endogenous polypeptide relative to seeds that do not contain or express said first nucleic acid.

- 23. (Original) The method of claim 22, wherein each said promoter for said one or more activator nucleic acids is an Arabidopsis LEC1 promoter.
- 24. (Original) The method of claim 22, wherein said plurality of first plants and said plurality of second plants are randomly interplanted.
- 25. (Original) The method of claim 22, wherein said sequence causing seed infertility encodes a seed infertility polypeptide.
- 26. (Previously presented) The method of claim 27, further comprising the step of extracting said preselected polypeptide from said seeds.
- 27. (Currently amended) A method for making a polypeptide, said method comprising:
- a) permitting a plurality of first plants to be pollinated by a plurality of second plants, each of said first plants being male-sterile due to cytoplasmic male sterility, nuclear male sterility or genetic male sterility comprising: i) a first nucleic acid comprising a first

Serial No. : 10/667,295

Filed: September 17, 2003

Page : 6 of 18

transcription activator recognition site and a first promoter, said first recognition site and said first promoter operably linked to a nucleic acid encoding a preselected industrial or pharmaceutical polypeptide; and ii) a second nucleic acid comprising a second transcription activator recognition site and a second promoter, said second recognition site and said second promoter operably linked to a sequence causing seed infertility, each of said second plants comprising at least one activator nucleic acid encoding at least one transcription activator that binds to at least one of said recognition sites, each said at least one transcription activator nucleic acid having a promoter operably linked thereto; and

b) harvesting seeds from said plurality of first plants, wherein said seeds are infertile and have a statistically significant increase in said preselected polypeptide relative to seeds that do not contain or express said first nucleic acid.

## 28. (Currently amended) An article of manufacture comprising:

- a) a container;
- b) a first type of seeds within said container, said first type of seeds comprising at least one first nucleic acid comprising: i) a first transcription activator recognition site and a first promoter, said first recognition site and said first promoter operably linked to a sequence encoding a preselected industrial or pharmaceutical polypeptide; and ii) a second transcription activator recognition site and a second promoter, said second recognition site and said second promoter operably linked to a sequence causing seed infertility, wherein plants grown from said first type of seeds are male-sterile due to cytoplasmic male sterility, nuclear male sterility or genetic male sterility; and
- c) a second type of seeds within said container, said second type of seeds comprising at least one activator nucleic acid encoding at least one transcription activator that binds to at least one of said recognition sites, each said at least one transcription activator having a promoter operably linked thereto, wherein plants grown from said second type of seeds are male-fertile.

## 29. (Canceled)

Serial No. : 10/667,295

Filed: September 17, 2003

Page : 7 of 18

30. (Original) The article of claim 28, wherein the ratio of said first type of seeds to said second type of seeds is about 70:30 or greater.

- 31. (Original) The article of claim 28, wherein said at least one first nucleic acid comprises a nucleic acid comprising said first transcription activator recognition site, said first promoter and said sequence to be transcribed, and a different nucleic acid comprising said second transcription activator recognition site, said second promoter and a seed infertility polypeptide coding sequence.
- 32. (Original) The article of claim 28, wherein said at least one activator nucleic acid encodes a transcription activator that binds to said first recognition site, and a different transcription activator that binds to said second recognition site.
- 33. (Original) The article of claim 32, wherein said promoter for said transcription activator that binds said first recognition site is a seed-specific promoter and said promoter for said transcription activator that binds to said second recognition site is a maize ubiquitin promoter.
- 34. (Original) The article of claim 28, wherein said first and said second types of seeds are dicotyledonous seeds.
- 35. (Original) The article of claim 28, wherein said first and said second types of seeds are monocotyledonous seeds.
- 36. (Original) The article of claim 28, wherein said first type of seeds are cytoplasmically male sterile.
  - 37. (Withdrawn) A nucleic acid construct comprising:
- a) a first transcription activator recognition site and a first promoter, said first recognition site and said first promoter operably linked to a sequence to be transcribed; and

Serial No.: 10/667,295

Filed: September 17, 2003

Page : 8 of 18

b) a second transcription activator recognition site and a second promoter, said second recognition site and said second promoter operably linked to a sequence causing seed infertility.

- 38. (Withdrawn) The nucleic acid construct of claim 37, wherein said sequence causing seed infertility is transcribed into a FIE antagonist.
- 39. (Withdrawn) The nucleic acid construct of claim 37, wherein said FIE antagonist is an antisense RNA.
- 40. (Withdrawn) The nucleic acid construct of claim 37, wherein said FIE antagonist is a ribozyme
- 41. (Withdrawn) The nucleic acid construct of claim 37, wherein said FIE antagonist is a chimeric polypeptide comprising a polypeptide segment exhibiting histone acetyltransferase activity fused to a polypeptide segment exhibiting activity of a subunit of a chromatin-associated protein complex having histone deacetylase activity.
- 42. (Withdrawn) The nucleic acid construct of claim 37, wherein said sequence to be transcribed encodes a preselected polypeptide.
- 43. (Withdrawn) The nucleic acid construct of claim 42, wherein said preselected polypeptide is an antibody.
- 44. (Withdrawn) The nucleic acid construct of claim 42, wherein said preselected polypeptide has immunogenic activity in a mammal.
- 45. (Withdrawn) The nucleic acid construct of claim 42, wherein said preselected polypeptide is an enzyme.

Serial No.: 10/667,295

Filed: September 17, 2003

Page : 9 of 18

46. (Withdrawn) The nucleic acid construct of claim 45, wherein said preselected polypeptide is glucose-6-phosphate dehydrogenase.

- 47. (Withdrawn) The nucleic acid construct of claim 45, wherein said preselected polypeptide is alpha-amylase.
- 48. (Withdrawn) The nucleic acid construct of claim 37, wherein said sequence causing seed infertility encodes ANT.
- 49. (Withdrawn) The nucleic acid construct of claim 37, wherein said sequence causing seed infertility encodes LEC1.
  - 50. (Currently amended) A male-sterile plant comprising:
- a) a first nucleic acid comprising a first transcription activator recognition site and a first promoter, said first recognition site and said first promoter operably linked to a sequence encoding a preselected industrial or pharmaceutical polypeptide; and
- b) a second nucleic acid comprising a second transcription activator recognition site and a second promoter, said second recognition site and said second promoter operably linked to a sequence causing seed infertility, wherein said plant is male-sterile due to cytoplasmic male sterility, nuclear male sterility or genetic male sterility.
  - 51. (Canceled)
- 52. (Original) The plant of claim 50, wherein said plant is cytoplasmically male sterile.
- 53. (Currently amended) The plant of claim 50, wherein said plant is male-sterile due to nucleargenetic male sterility.

Serial No. : 10/667,295

Filed: September 17, 2003

Page : 10 of 18

54. (Original) The plant of claim 50, wherein said plant is genetically male sterile.

- 55. (Original) The plant of claim 50, wherein said first and second nucleic acids are a single nucleic acid molecule.
  - 56. (Original) The plant of claim 50, wherein said plant is a dicotyledonous plant.
- 57. (Original) The plant of claim 50, wherein said plant is a monocotyledonous plant.
- 58. (Previously presented) The plant of claim 50, wherein said preselected industrial or pharmaceutical polypeptide is an enzyme.
  - 59. (Previously presented) The plant of claim 50, further comprising a third nucleic acid comprising a coding sequence for a transcription activator that binds said first recognition site, operably linked to a constitutive promoter.
- 60. (Previously presented) The plant of claim 50, further comprising a fourth nucleic acid comprising a coding sequence for a transcription activator that binds to said second recognition site, operably linked to a constitutive promoter.
- 61. (Previously presented) The plant of claim 56, wherein said plant is a *Glycine* plant.
- 62. (Previously presented) The plant of claim 57, wherein said plant is a *Panicum* plant.
- 63. (Previously presented) The plant of claim 57, wherein said plant is a Sorghum plant.

Applicant : Peter Mascia Serial No. : 10/667,295

Filed: September 17, 2003

Page : 11 of 18

64. (New) The method of claim 22, wherein said plant is cytoplasmically male sterile.

- 65. (New) The method of claim 22, wherein said plant is male-sterile due to genetic male sterility.
- 66. (New) The method of claim 27, wherein said plurality of first plants is cytoplasmically male sterile.
- 67. (New) The method of claim 27, wherein said plurality of first plants is malesterile due to genetic male sterility.
- 68. (New) The article of manufacture of claim 28, wherein said plants grown from said first type of seeds are cytoplasmically male sterile.
- 69. (New) The article of manufacture of claim 28, wherein said plants grown from said first type of seeds are male-sterile due to genetic male sterility.